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Further Experiments With the Iowa Air Blast Seed Separator for the Analysis of Small-Seeded Grasses

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BOTANY AND PLANT PATHOLOGY SECTION
FARM CROPS SUBSECTION
AGRONOMY SECTION

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CONTENTS

SUMMARY

The results of the experiments to determine percentages of pure seed of redtop, *Agrostis alba*, and Kentucky bluegrass, *Poa pratensis*, may be summarized as follows:

1. Separation of immature, undeveloped or empty florets from heavy (pure) seed of both redtop and bluegrass can be accomplished in a reasonably uniform manner by the use of the Iowa air blast seed separator. Some ergotized and smutted florets and other foreign material remain in the heavy fraction and must be removed by hand.

2. The separator unit without the vernier scale on the valve dial can be set accurately to ½ degree. A valve equipped with the vernier can be adjusted accurately to 1/10 degree. The finer the adjustment the greater the precision.

3. At any given dial reading a fairly uniform number of germinable seeds is removed with the light-weight fraction, but the valve opening can be so adjusted that the number of such seeds is relatively small and has little effect on the percentage of pure seed. The net result of this method of testing grass seeds is that not only are the pure seed fractions uniform, but the index value (purity $\times$ germination) is greater than when immature and undeveloped florets difficult to classify are retained with the pure seed.

4. As the size of working sample of either redtop or bluegrass seed is reduced below 1 gram, the variance of the replicates increases more or less proportionately, and although the differences in percentage of purity of a given set of replicates may not be more than would be expected from homogeneous samples, the differences do become greater among small sub-samples than are permissible for practical application.

5. Half-gram sub-samples of redtop seed may be substituted for gram samples to determine percentages of pure seed, crop seed, weed seed and inert matter with an important reduction in time required to complete the analysis. It may even be possible to reduce the weight to ¼-gram fractions.

6. Tenth-gram sub-samples of bluegrass seed whether analyzed by the Iowa or binocular methods are too small for use at present in seed testing work, because

the differences among replicates are too great for practical use in the evaluation of seed lots. There is some indication that percentages of pure seed obtained by the binocular method are higher on the average than obtained by the uniform method.

7. A uniform method of analyzing bluegrass seed can enable shippers and buyers to evaluate mixing, bulking and sampling methods.

Further Experiments With the Iowa Air Blast Seed Separator for the Analysis of Small-Seeded Grasses[1]

By Ewert Åberg, R. H. Porter and Wayne A. Robbins[2]

In recent years attempts have been made in Europe and North America to improve seed laboratory machines used for the separation of chaff from heavy seeds in grasses. The most important European contribution is probably the Gilchrist seed separator[3] used at the seed testing station in Edinburgh, Scotland. The Holland (Leendertz) separator has some merit, but its chief weakness as shown by Porter (7) was the inconstant speed of the motor which made it impossible to deliver the same volume of air through the separator tube each time the valve was opened to a given point. Replacement of the motor by one that operated at constant speed was found by Brown and Porter (1) to correct the defect reasonably well. The use of bolting cloth in place of a metal screen is also a disadvantage. Following the initial work Porter (8) described a new seed separator equipped with a combined motor and fan unit of standard specifications which made uniform separations of bluegrass, redtop and orchard grass samples either by repeated blowings of the same sub-sample or by a single blowing for each of many replicate sub-samples. He also employed synthetic samples to check the accuracy and uniformity of separation. These results were obtained with samples from the same lot and indicated that for any given lot of seed it was possible to obtain uniform results if care was used in reading the dial opening on the valve or the manometer which indicated pressure in the compression chamber. It is noteworthy that in his experiments Porter (8) showed that if the weight of sample varied from ½ to 2 grams of bluegrass seed, the dial reading was more dependable than pressure as measured in the compression chamber, and further that pressure as measured in the blowing tube was about as reliable as the dial reading. He showed, however, that with samples having a low weight per bushel the amount of ger-

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[1] Project 86 of the Iowa Agricultural Experiment Station.

[2] Research associate in Botany and Farm Crops 1941-42, while on leave from the Agricultural College, Uppsala, Sweden; research professor in agronomy; formerly research assistant; respectively. All in the Iowa Agricultural Experiment Station, Ames, Iowa.

[3] The senior author observed this seed separator in operation at the Seed Testing Station in Edinburgh in 1938 and got the impression that it was superior to other European seed separators.

minable seed removed at a given opening was greater than with samples having a high weight per bushel.

Leggatt (3, 4) developed the Ottawa seed separator equipped with a manometer and a gate opening to regulate the amount of air that entered the chamber. He found that these two devices were convenient as a check on each other, but that of the two, the gate opening was most reliable as a criterion for blowing samples of bluegrass. Subsequently, Leggatt (5) employed a standard synthetic sample to calibrate his machine each day and thus determine the proper gate opening for that day which varied slightly with air humidity.

Laboratory tests by the senior author using the Iowa air blast seed separator in the Iowa State College Seed Laboratory in the winter of 1941-1942 indicated that manometer readings were somewhat difficult to make accurately, and that the dial which was graduated only to even degrees, not to fractions, could be read accurately only when the needle was set at intervals of 1 or ½ degree. To determine the degree of uniformity with which one could operate the seed separator some tests were undertaken with seed of redtop, *Agrostis alba* L., and bluegrass, *Poa pratensis* L., using (1) samples of different weight and quality, (2) replicate blowings of the same sample, (3) pressure in the blowing tube as the standard criterion and (4) dial readings as the measure of pressure. In addition some tests were made with the seed separator equipped with a vernier scale on the valve which made it possible to regulate the air escape opening very accurately to 1/10 of a degree.

It is the purpose of this bulletin to report the results of these respective tests and attempt further to evaluate the application to seed testing of the new concept of pure seed as has been recently described by Porter and Leggatt (9).

EXPERIMENTS WITH REDTOP

A series of experiments with seed of redtop were undertaken to determine (a) if one blowing of ½-gram samples of redtop seed for 3 minutes at a compression chamber reading of 10 mm. on the manometer board or a standard dial reading could be used to provide an index value comparable to the "Climax Blowing Point" as described by Leggatt (5) and (b) if the size of sample could be reduced to ½ gram as proposed by Porter (8), or even to a smaller size.

For experiment 1, six lots of redtop seed were selected with percentages of pure seed ranging from 88 to 96. From each lot three sub-samples of 1 gram, three of ½ gram and three of 1/5 gram were obtained. Each sub-sample was blown 3 minutes at a dial reading of 35.5 which had been previously determined as the opening at which the compression chamber reading was 10 mm. when half-gram sub-samples were used. As was expected and as illustrated in table 3 the compression chamber reading was not 10 mm. when the 1-gram or 1/5-gram portions were blown at a dial reading of 35.5.

Following each blowing the weights of the heavy and light fractions were determined after which the heavy fraction was further separated by hand into pure seed, weed seeds, other crop seed and other foreign material including ergotized and smutted florets. All of the light fraction removed by air pressure was classed as inert and included with other foreign material removed by hand from the heavy fraction. Percentages by weight of heavy, pure seed, weed seed and crop seed were determined for each sub-sample. The data obtained are presented in table 1, and statistical evaluation of the data follows.

In the first section of table 1 in which the blowings and the analyses are based on 1 gram, a Chi-square test of the data showing percent heavy indicates that the differences between the replicates of 81345, 81856 and 81159 are significant, whereas the differences for the other three samples are not significant. If we consider the data for percentages of pure seed, weed seed and crop seed it is found that only for samples 81345 and 83126 are differences between replicates of pure seed percentages significant; for sample 83126 the differences in percentage of weed seed are significant and for sample 78594 the differences in percentage of crop seed are significant. All other differences are not significant.

An examination and analysis of all the data where ½-gram sub-samples were used shows that no significant differences occurred between the percentages of three replicates except for sample 78594, and that only in percentages of pure seed.

Analysis of all the data where ⅕-gram sub-samples were used shows that only for sample 78594, both in percentages of weed and crop seed, are the differences significant.

The evaluation of data involving sub-samples with different weights, and therefore a different number of particles (11,000 for 1 gram, 5,500 for ½ gram and 2,200 for ⅕ gram of redtop) permits larger differences between replicates of small than between large sub-samples without changing the degree of probability. For example, the differences in percentages of heavy material for sample 81159 using 1-gram sub-samples are significant, yet the difference in ex-

TABLE 1. PERCENT HEAVY FRACTIONS FROM BLOWINGS OF REDTOP AND PERCENT PURE SEED, WEED SEED AND CROP SEED IN TRIPLICATE SUB-SAMPLES OBTAINED WITH IOWA AIR BLAST SEED SEPARATOR USING A CONSTANT DIAL READING OF 35.5.

Sample no.	Approx. weight sub-sample	Percent by replicates: Pct. heavy by replicates			Pure seed				Weed seed				Crop seed				Anal. time min.
		1	2	3	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	
81345	1 gram	98.1	98.7	97.9*	96.6	96.2	95.6	96.13*	0.4	0.2	0.3	0.3	0.1	—	—	0.03	95
81856	1 gram	98.3	98.8	99.2*	95.6	95.9	95.7	95.73	0.1	0.1	—	0.07	—	—	—	—	107
81159	1 gram	93.6	93.2	94.3*	91.4	91.0	91.5	91.30	1.1	1.0	1.2	1.1	—	—	—	—	113
83126	1 gram	94.4	94.3	94.0	91.4	91.5	90.6	91.17*	0.5	0.9	0.6	0.67*	—	—	—	—	101
80760	1 gram	95.6	95.6	95.7	89.6	89.5	88.8	89.30	0.4	0.6	0.6	0.53	—	—	—	—	140
78594	1 gram	96.9	97.1	96.5	89.4	88.5	89.3	89.07	0.4	0.6	0.6	0.53	0.3	0.4	0.9	0.53*	155
81345	½ gram	98.6	98.4	98.4	96.5	96.7	96.4	96.53	0.5	0.4	0.5	0.47	—	—	—	—	45
81856	½ gram	99.4	99.5	99.4	96.0	96.1	96.3	96.33	0.1	—	0.1	0.07	—	—	—	—	65
81159	½ gram	95.3	94.8	94.7	92.3	91.7	91.7	91.90	1.4	1.0	1.3	1.23	—	—	—	—	73
83126	½ gram	93.3	93.2	92.4	90.7	90.7	89.8	90.40	0.8	0.6	0.7	0.7	0.1	0.1	0.1	0.1	60
80760	½ gram	96.1	95.9	95.9	87.1	88.2	88.4	87.90	0.4	0.2	0.4	0.33	—	—	—	—	97
78594	½ gram	96.9	97.0	97.0	90.1	88.5	89.2	89.27*	0.5	0.5	0.6	0.53	0.6	0.8	0.5	0.63	98
81345	1/5 gram	97.8	98.3	98.0	95.9	96.8	96.6	96.30	0.4	0.5	0.3	0.4	0.4	—	—	0.13	30
81856	1/5 gram	99.5	99.4	99.4	96.3	95.9	96.1	96.10	—	—	—	—	—	—	—	—	25
81159	1/5 gram	95.4	94.6	95.8	91.2	89.9	91.8	90.97	1.7	1.9	1.2	1.6	—	—	—	—	33
83126	1/5 gram	95.0	95.3	95.0	91.7	90.9	91.4	91.33	0.7	0.8	0.9	0.8	—	—	—	—	32
80760	1/5 gram	96.3	96.6	96.7	88.1	88.1	87.4	87.87	0.4	0.4	0.8	0.53	—	—	—	—	43
78594	1/5 gram	98.1	97.8	98.3	89.4	89.9	89.3	89.53	1.2	0.7	0.6	0.83*	1.5	0.4	0.7	0.87*	40

* Differences between three replicates significant.

tremes is 1.1, whereas when 1/5-gram sub-samples were used the difference is 1.2, yet the differences between the three replicates are not significant. Taken as a whole the data show that:

1. Blowing triplicate sub-samples of redtop seed using 1, 1/2 and 1/5 grams, respectively, per sub-sample with the Iowa air blast seed separator at a fixed dial reading for 3 minutes gave reasonably uniform percentages of heavy material, pure seed, crop seed and weed seed within each set of triplicates;
2. The mean percentages of pure seed for each sample regardless of sub-sample size were similar with the exception of sample 80760 in which case the mean percentage of pure seed was significantly higher for 1-gram than for either 1/2- or 1/5-gram sub-samples. The mean percentages of heavy material, weed seed and crop seed were more variable, the results with 1/5-gram sub-samples showing the greatest variation;
3. More uniformity was achieved with 1/2-gram sub-samples than with either 1- or 1/5-gram portions;
4. Percentages of crop seed and weed seed were more variable when 1/5-gram sub-samples were used than for either 1/2- or 1-gram, and in three cases were greater than is permitted under the tolerance formula as used by the Association of Official Seed Analysts and in the enforcement of the Federal Seed Act;
5. The average time required to analyze sub-samples of different weights was 118 minutes for 1 gram, 73 for 1/2 gram and 32 for 1/5 gram.

Following the experiments with the seed separator the pure seed and the inert fractions from replication 3 of each sub-sample were tested for germination. The percentage germination of each pure seed fraction, the pure live seed and the germinated seeds in the inert fraction expressed in number and percent of the total germinating seeds are shown in table 2. The thousand-seed weight of the samples was used as a basis for determining the number of seeds per sample.

The data in column 6 of table 2 show that the number of germinable seeds in the inert fraction differed greatly among samples. Since these germinable seeds were removed by the seed separator and not by hand, one might conclude that the separator did not operate in a uniform manner. This could be determined more correctly if each member of the triplicate sub-samples from each sample had been tested or if each sub-sample were exactly the same weight. One could expect individual lots of redtop seed to differ in the

TABLE 2. PERCENTAGE GERMINATION OF PURE SEED FRACTIONS AND NUMBER OF GERMINABLE SEEDS IN INERT FRACTIONS OF REDTOP SEED. IOWA 1941.

Sample no.	Approx. weight sample	No. pure seeds	Pct. germination	Germinable seed in			Pct. germinable seeds in inert are of total
				Pure s.	Inert	Total	
81345	1 gram	11365	95.33	10834	156	10990	1.42
81856	1 gram	10575	95.42	10091	66	10157	0.65
81159	1 gram	10816	88.83	9608	224	9832	2.28
83126	1 gram	10517	92.67	9746	156	9902	1.58
80760	1 gram	11459	92.00	10542	143	10685	1.31
78594	1 gram	10538	78.42	8264	258	8522	3.03
81345	½ gram	5833	95.33	5561	57	5618	1.01
81856	½ gram	5659	95.42	5400	17	5417	.31
81159	½ gram	6000	88.83	5330	75	5405	1.39
83126	½ gram	5035	92.67	4666	108	4774	2.26
80760	½ gram	5683	92.00	5228	174	5402	3.22
78594	½ gram	5728	78.42	4492	162	4654	3.48
81345	1/5 gram	2383	95.33	2272	14	2286	0.61
81856	1/5 gram	2091	95.42	1995	6	2001	0.30
81159	1/5 gram	2292	88.83	2036	37	2073	1.78
83126	1/5 gram	1893	92.67	1754	17	1771	0.96
80760	1/5 gram	2251	92.00	2071	35	2106	1.66
78594	1/5 gram	1963	78.42	1539	28	1567	1.79

size, weight, maturity and specific gravity of certain seeds, hence the separator could hardly be expected to remove a similar number of viable seeds from each lot. One ought to expect, however, that for each sample or lot there would be proportionately fewer viable seeds removed in ½- and ⅕-gram sub-samples than in 1-gram sub-samples. The numbers are less, but not proportionately so. This may well be accounted for by the fact that the dial could not be set accurately at 35.5 each time. It also seems probable that when the ⅕-gram sub-samples are blown there is so much less interference among particles as they are lifted by air pressure into the blowing tube, that a smaller percentage is removed. Whatever the cause, it is evident that the seed separator when set at a dial reading of 35.5 removed a considerable number of germinable seeds, but not in a highly uniform manner. Possibly a more refined method of standardizing the opening of the dial is needed.

RELATION OF DIAL READING TO PRESSURE IN THE COMPRESSION CHAMBER AND BLOWING TUBE

In 1938 Porter (8) conducted one experiment with bluegrass seed in which he found that when the weight of sample was reduced from 2 grams to ½ gram the pressure in the compression chamber was unreliable as a criterion for separating chaff from heavy seed. On the other hand, if the dial reading was held constant, separation was uniform for samples of different weights and pressure in the blowing tube was also constant.

Two sets of experiments were undertaken in this study with redtop seed. The first one was designed to compare the dial readings with pressure readings in the compression chamber. The second attempted to determine the interrelationship of the three possible criteria for establishing a standard procedure.

For the first set of experiments, six samples of redtop seed were selected with purity percentages ranging from 81 to 95 percent. From each sample one sub-sample of 1 gram, one of ½ gram and one of ⅕ gram were obtained. Preliminary tests had shown that the pressure in the chamber should be approximately 10.5, 10 and 9 mm., respectively, for sub-samples of 1, ½ and ⅕ grams in weight. Accordingly, the experiment was so designed that each sub-sample was blown for 3 minutes at the predetermined manometer reading after which the heavy and light fractions were weighed and the percent heavy calculated. The fractions were then recombined and reblown twice more making a total of three blowings for the sub-sample. During each period of blowing, the readings on the dial were recorded. The recombined sub-sample was then blown three times with the dial reading at 35.5 as the constant, and the manometer readings in millimeters in the compression chamber were recorded.

It is unnecessary to record the data for all six samples because the results were similar. Table 3 shows the data obtained from two samples. To properly evaluate the data in table 3 it is necessary to recall that, with the particular separator unit, readings could be made accurately on the dial only to even degrees and on the manometer only to half-millimeters, hence all other recordings are only approximate.

For sample 80763 it should be noted that when the first 1-gram sub-sample was blown with the manometer reading at 10.5, the dial readings in repeated blowings averaged slightly above 35.5. When the second 1-gram sub-sample was blown with the dial reading constant at 35.5 the manometer readings averaged 10.5. Since manometer readings were made with more difficulty than dial readings they are in general considered less accurate.

The results with the ½- and ⅕-gram sub-samples show that the mean percent heavy was higher for the second than for the first which is as would be expected, because the lower the dial reading the higher the percent heavy material. This relationship did not hold for the 1-gram sub-samples. For sample 84380 the results are more striking in that the percent heavy was much less than for sample 80763, and the differences in dial readings for each pair of sub-samples were greater.

With two exceptions the results of repeated blowings of the same sub-sample were reasonably uniform. They ap-

TABLE 3. DATA FROM BLOWING TESTS WITH REDTOP SEED USING DIAL READINGS AS AGAINST MANOMETRIC PRESSURE IN THE COMPRESSION CHAMBER—IOWA AIR BLAST SEED SEPARATOR. AMES, IOWA, 1941.

Sample no.	Weight	Sub-sample	Basic criteria *	Dial or manometer reading				Percent heavy			
				1	2	3	Mean	1	2	3	Mean
80763	1 gram	a	10.5 mm.	35.6°	35.6°	35.8°	35.7°	94.7	96.0	96.3	95.7
	1 gram	b	35.5°	10.3 mm.	10.4	10.8	10.5	95.0	95.3	95.6	95.3
	½ gram	a	10 mm.	36.0°	36.1°	36.1°	36.1°	95.7	95.2	95.6	95.5
	½ gram	b	35.5°	9.5 mm.	9.5	9.8	9.6	96.3	96.0	95.7	96.0
	1/5 gram	a	9 mm.	36.0°	35.9°	35.9°	35.9°	96.0	95.4	95.5	95.6
	1/5 gram	b	35.5°	8.8 mm.	9.0	8.8	8.9	96.6	95.9	96.0	96.2
84380	1 gram	a	10.5 mm.	36.1°	35.9°	35.9°	36.0°	81.6	82.2	82.3	82.0
	1 gram	b	35.5°	10 mm.	10	10	10	85.3	86.1	83.8	85.1
	½ gram	a	10 mm.	36.1°	36.2°	36.2°	36.2°	81.6	82.4	81.9	82.0
	½ gram	b	35.5°	9.0 mm.	9.1	9.2	9.1	83.9	83.9	83.4	83.7
	1/5 gram	a	9 mm.	35.9°	36.2°	36.1°	36.1°	82.8	82.8	83.2	82.9
	1/5 gram	b	35.5°	8.6 mm.	8.5	8.5	8.5	83.6	84.2	84.5	84.1

* Basic criteria a = Standard setting by manometric pressure in compression chamber for each of three blowings per sub-sample with corresponding dial readings for each blowing.

b = Standard setting by dial for each of three blowings per sub-sample with corresponding pressure readings in compression chamber for each blowing.

pear to be of some significance, particularly when the difficulties of making fine mechanical adjustments with the separator are considered as well as possible loss of small particles from sub-samples of ½ to ⅕ gram when they were separated and recombined.

The main conclusion that may be drawn from these experiments is that for a given weight of sample, dependence on either the dial reading or the manometer reading in the compression chamber gives equally uniform percentages of heavy seed provided one does not attempt to make a finer adjustment than the scale or dial graduations will permit. If the manometer readings are used as the criteria it is necessary to know the proper pressure for a given size of sample, hence the dial reading must be considered more dependable.

INTERRELATIONSHIP OF DIAL AND MANOMETER READINGS

To determine the interrelationship of dial readings and manometer readings in the compression chamber and tube, two experiments were planned and executed.

In the first one three lots of redtop seed were selected and from each lot three sub-samples weighing approximately 1 gram, three weighing ½ gram, and three weighing ⅕ gram, respectively, were subdivided. Each sub-sample was then blown and recombined as follows: Using a predetermined manometer reading in the compression chamber a sub-sample (for example 1 gram) was blown for 3 minutes, the heavy and light portions weighed and recombined and the dial reading recorded. The sub-sample was then reblown using the previously recorded dial reading as the criterion, and the pressure in the tube was recorded. Again the sub-sample was recombined and blown using 35.5 on the dial as the basis, and the pressure in the chamber was recorded. Finally the sub-sample was blown at a dial reading of 35.5, and the pressure in the tube was recorded. All together each sub-sample was blown and recombined four times, which presumably resulted in some loss of particles as well as changes in moisture content.

It is unnecessary to record here the data for all three samples; those for one sample, no. 84380, are given in table 4. They are fairly representative of the other two samples.

The data in table 4 require explanation and interpretation. For example, it is clear that as the weight of sample was reduced, the pressure in the compression chamber was lowered even though the dial reading remained nearly constant. Furthermore, the percentages of heavy seed with either 1- or ½-gram sub-samples were similar with a dial at 35.9 to 36.2, but with ⅕-gram sub-samples the percent-

TABLE 4. DATA FROM BLOWING TESTS WITH REDTOP SEED SHOWING INTERRELATIONSHIP OF DIAL READINGS AND PRESSURE IN CHAMBER AND TUBE. IOWA AIR BLAST SEED SEPARATOR—1941. SAMPLE NO. 84380.

Sample: Weight	Sample: No.	Chamber pressure constant	Dial reading	Pct. heavy	Previous dial reading	Tube pressure	Pct. heavy	Dial constant	Chamber pressure	Pct. heavy	Dial constant	Tube pressure	Pct. heavy
1 gram	1	10.5	36.0°	82.0	36.0°	4.6	82.2	35.5	9.5	83.5	35.5	4.0	83.5
1 gram	2	10.5	36.0°	80.7	36.0°	4.6	80.8	35.5	9.3	82.4	35.5	4.1	82.6
1 gram	3	10.5	35.9°	81.1	35.9°	4.9	81.3	35.5	9.7	83.2	35.5	4.0	82.4
½ gram	1	10	36.2°	80.9	36.2°	5.0	82.4	35.5	8.7	82.7	35.5	4.0	82.5
½ gram	2	10	36.2°	81.9	36.2°	4.9	81.6	35.5	7.6	82.1	35.5	4.1	82.6
½ gram	3	10	36.2°	82.1	36.2°	5.0	82.4	35.5	8.1	83.1	35.5	4.1	83.0
1/5 gram	1	9	36.0°	83.1	36.0°	5.0	83.9	35.5	8.0	84.7	35.5	4.1	85.0
1/5 gram	2	9	36.0°	82.3	36.0°	5.0	82.7	35.5	8.3	84.2	35.5	4.1	84.6
1/5 gram	3	9	36.0°	83.1	36.0°	5.0	83.4	35.5	8.0	84.5	35.5	4.0	84.4

ages averaged higher, indicating that when the sample size is that small a slightly higher dial reading is required for uniform results. In addition it may be noted that with the exception of samples 1 and 2 (1 gram weight) the tube pressure was nearly constant with dial readings between 35.9 and 36.2 regardless of weight of sub-sample.

The data in the right half of table 4 are of particular interest because all the dial readings used as the basic criteria were 35.5, and the corresponding pressures in the tube were remarkably uniform. Furthermore, the figures for percent heavy in columns 10 and 13 of the table for any one sub-sample represent two blowings of the same sub-sample. The results are remarkably uniform, and if we consider the six percentages for the 1- and the ½-gram samples in either columns 11 or 14 (representing six sub-samples from sample 84380) we note a normal variation in sampling.

In the second experiment of this series the machine was equipped with two manometers so that pressure in millimeters could be read simultaneously in the compression chamber and blowing tube. Seed of three samples of redtop was obtained, and from each sample four sub-samples each of 1 gram, ½ gram and ⅕ gram, respectively, were subdivided. Each sub-sample was blown and recombined twice so that three determinations of percent heavy seed were made. The pressure in the tube was taken as the constant for each three blowings of the first sub-sample. As soon as the separator was in operation the pressure in the chamber and the dial reading were each recorded. The second sub-sample of a given weight was treated in the same manner except that the tube pressure was raised ½ millimeter.

The data obtained with the blowing tests of samples 81159 and 80760 are given in table 5. As was noted in table 4 there is a reasonably constant relationship between millimeters of pressure in the tube and the dial readings in degrees for replicate blowings of the same sub-sample. Of most importance, however, is the further demonstration that when a given sub-sample is blown, recombined and blown twice again the percentage heavy material is nearly the same for each blowing, even with portions as small as ⅕ gram. These results indicate a reasonable uniformity in the performance of the separator and normal variation among sub-samples of a given lot, at least for 1-gram and ½-gram portions blown with the same pressure in the tube.

COMPARATIVE ANALYSES OF 1-, ½- AND ¼-GRAM SUB-SAMPLES OF REDTOP SEED

In 1937 Porter (11) as chairman of Region No. 2 of the Association of Official Seed Analysts conducted a referee test

TABLE 5. PERCENT HEAVY SEED OF REDTOP OBTAINED FROM BLOWING TESTS BASED ON TUBE PRESSURE AS THE CONSTANT WITH RECORDINGS OF CHAMBER PRESSURE AND DIAL READINGS. IOWA AIR BLAST SEED SEPARATOR—1941.

Sample		Replicate	Tube pressure	Chamber pressure by repetition			Dial reading by repetition			Percent heavy		
No.	Wt.			1	2	3	1	2	3	1	2	3
81159	1 gram	1	4.0	10.3	10.1	10.1	34.9	35.0	35.0	95.0	94.9	94.4
	1 gram	2	4.5	11.3	11.3	11.0	35.8	35.8	35.8	93.1	92.9	92.8
	1 gram	3	5.0	11.3	11.7	11.7	36.3	36.3	36.3	93.2	92.8	93.0
	1 gram	4	5.5	12.2	12.2	12.8	37.0	36.8	37.0	91.4	91.2	91.4
	½ gram	1	4.0	8.9	8.7	8.4	35.0	35.1	35.1	95.4	95.7	95.8
	½ gram	2	4.5	9.1	9.1	9.4	35.4	35.7	35.8	93.7	93.8	94.3
	½ gram	3	5.0	9.8	10.1	9.6	36.2	36.5	36.2	92.2	92.4	92.0
	½ gram	4	5.5	10.5	10.3	10.8	36.9	36.8	36.9	90.7	90.4	90.8
	1/5 gram	1	4.0	7.5	7.0	7.0	34.8	34.9	34.9	96.6	96.4	96.6
	1/5 gram	2	4.5	7.5	8.0	8.0	35.2	35.2	35.2	94.4	94.4	94.0
	1/5 gram	3	5.0	8.7	8.9	8.9	36.0	36.1	36.0	94.0	93.6	93.8
	1/5 gram	4	5.5	9.4	9.4	9.6	36.5	36.8	36.7	92.3	92.9	92.8
80760	1 gram	1	4.0	9.4	9.6	9.8	34.7	34.8	34.6	96.0	96.3	96.0
	1 gram	2	4.5	9.6	9.6	9.8	35.1	35.1	35.0	95.7	95.8	95.8
	1 gram	3	5.0	9.8	10.1	9.8	35.8	36.0	35.9	94.6	95.3	95.1
	1 gram	4	5.5	10.8	10.8	10.8	36.2	36.2	36.2	92.4	92.0	92.6
	½ gram	1	4.0	8.7	7.7	7.7	35.0	35.0	35.0	96.6	96.2	96.8
	½ gram	2	4.5	8.7	8.9	8.9	35.3	35.5	35.5	95.2	95.5	95.6
	½ gram	3	5.0	9.8	9.8	9.8	36.2	36.4	36.2	94.3	93.8	94.3
	½ gram	4	5.5	10.3	10.5	10.3	36.5	36.7	36.7	91.2	90.6	90.6
	1/5 gram	1	4.0	7.0	7.5	7.3	34.9	35.0	34.9	96.6	96.5	96.9
	1/5 gram	2	4.5	8.2	8.2	8.4	35.3	35.2	35.4	96.4	96.1	96.5
	1/5 gram	3	5.0	9.4	9.1	8.9	36.2	36.2	36.2	95.0	94.3	94.3
	1/5 gram	4	5.5	9.6	9.6	9.6	36.7	36.6	36.7	93.0	93.2	92.5

among laboratories of the region using 1-gram and ½-gram sub-samples of redtop seed. Samples were prepared by first subdividing a lot until several 1-gram sub-samples were obtained. Each was then subdivided to two ½-gram portions and each cooperating laboratory was requested to analyze each portion separately. The data were all compiled so that an evaluation could be made with ½-gram sub-samples or with 1-gram sub-samples by combining the weights obtained from each pair of ½-gram portions. The results indicated that for any one laboratory ½-gram sub-samples gave results comparable to 1-gram samples and within the range of natural variation. As between laboratories, differences were often too great, but they could be attributed to differences in classification of particles by analysts rather than to significant differences in samples. In 1938 Porter (8) published the results of analyzing four ½-gram sub-samples of redtop seed blown first at a standard pressure reading in the Iowa air blast seed separator. The percentages of pure seed, crop seed, weed seed and inert were uniform. He also conducted a similar test with 32 ½-gram samples of redtop seed from a given lot whose purity was 92 percent. Analysis of the percentages of the pure seed by the Chi-square test gave a probability value of .39 and germination tests in quadruplicate from eight pure seed fractions gave percentages with a probability value of .52. In 1941 the sub-committee of the Research Committee for the Analysis of Small Seeds (Assoc. of Off. Seed Anal.) (14) conducted a referee test with two samples of redtop and two of bent grass using ½-, 1- and 2-gram sub-samples. The conclusions reached were: (1) ½ gram appeared to be of sufficient size for the working sample of *Agrostis tenuis* Sibth., and (2) the results were considered insufficient for a definite recommendation concerning size of working sample for *Agrostis alba* and *Agrostis palustris* Huds.

For the particular experiment herein reported two lots of redtop seed were selected with purity percentages of about 93 and 83, respectively. The experiment was designed with three replications for each lot, and within each replication the following plan was used. Three 1-gram sub-samples were obtained by subdivision and one was analyzed as a unit, the second was divided into two ½-gram sub-samples and the third into ¼-gram sub-samples. The detailed analysis of each sub-sample was completed after blowing at a dial opening of 35. All pure seeds in the fraction removed by air pressure were removed by hand, then counted and weighed. An additional blowing of the recombined fractions was made with a dial opening of 36, and the number of pure

TABLE 6. WEIGHTS AND PERCENTAGES OF PURE SEED, CROP SEED, INERT MATERIAL AND WEED SEED FROM 1-, ½- AND ¼-GRAM SUB-SAMPLES OF REDTOP SEED. IOWA, 1941.

4750 Approx. wt. sub-sample	Time min.	Grams pure seed in fractions of			Percentages				No. pure seeds in light fraction at dial	
		Heavy	Light	Total	Pure seed	Crop seed	Inert	Weed seed	35	36
Replication 1										
1 gram	105	.8981	.0049	.9030	92.98	0.52	6.30	0.20	21	126
½ gram	60	.4307	.0040	.4347	92.86	0.26	6.79	0.09	14	99
½ gram	60	.3923	.0021	.3944	92.21	0.65	6.99	0.15	8	57
¼ gram	40	.2671	.0016	.2687	92.88	0.38	6.67	0.07	6	40
¼ gram	40	.2410	.0014	.2424	92.34	0.76	6.71	0.19	10	36
¼ gram	40	.2220	.0017	.2237	93.21	0.70	5.96	0.13	7	40
¼ gram	35	.1947	.0015	.1962	92.16	0.52	7.09	0.23	15	38
Replication 2										
1 gram	105	.9130	.0070	.9200	92.27	0.68	6.68	0.37	35	148
½ gram	60	.4936	.0022	.4958	92.90	0.24	6.62	0.24	16	68
½ gram	55	.4615	.0020	.4635	92.59	0.24	6.93	0.24	11	51
¼ gram	40	.2493	.0016	.2509	93.17	0.26	6.42	0.15	12	36
¼ gram	40	.2285	.0016	.2301	92.60	0.40	6.84	0.16	5	34
¼ gram	40	.2463	.0013	.2475	90.79	1.14	7.63	0.44	11	24
¼ gram	40	.2661	.0023	.2684	92.81	0.21	6.81	0.17	8	33
Replication 3										
1 gram	115	.9552	.0055	.9607	92.60	0.60	6.64	0.16	28	127
½ gram	65	.4882	.0026	.4908	92.38	0.77	6.81	0.04	14	65
½ gram	55	.4497	.0025	.4522	92.97	0.56	6.39	0.08	8	62
¼ gram	40	.2265	.0009	.2274	91.77	0.60	7.47	0.16	7	25
¼ gram	45	.2198	.0010	.2208	92.73	1.26	5.71	0.30	7	27
¼ gram	40	.2202	.0010	.2212	92.13	0.71	6.91	0.25	11	25
¼ gram	40	.1878	.0008	.1886	92.63	0.59	6.53	0.25	10	28

(Continued on next page.)

83419 Approx. wt. sub-sample	Time min.	Grams pure seed in fractions of			Percentages				No. pure seeds in light fraction at dial	
		Heavy	Light	Total	Pure seed	Crop seed	Inert	Weed seed	35	36
Replication 1										
1 gram	180	.7672	.0085	.7757	82.73	0.10	14.86	2.31	64	186
½ gram	95	.4209	.0048	.4257	82.48	0.19	14.67	2.66	18	108
½ gram	90	.3854	.0071	.3925	82.08	0.04	15.18	2.70	16	154
¼ gram	55	.2058	.0027	.2085	81.73	—	15.33	2.94	13	46
¼ gram	50	.1910	.0031	.1941	81.93	0.30	15.32	2.45	30	60
¼ gram	55	.2104	.0033	.2137	80.04	0.71	16.33	2.92	22	49
¼ gram	50	.1978	.0025	.2003	80.90	0.40	15.47	3.23	13	47
Replication 2										
1 gram	165	.7702	.0148	.7850	82.17	0.15	15.15	2.53	138	357
½ gram	80	.3982	.0068	.4050	82.65	0.06	14.68	2.61	31	177
½ gram	85	.3734	.0062	.3796	82.90	0.13	14.81	2.16	40	153
¼ gram	55	.2178	.0020	.2198	83.38	0.19	14.00	2.43	16	57
¼ gram	50	.1982	.0017	.1999	82.60	—	15.33	2.07	10	43
¼ gram	55	.2268	.0030	.2298	83.08	0.11	14.10	2.71	14	60
¼ gram	55	.2173	.0024	.2197	83.35	0.19	14.04	2.42	15	56
Replication 3										
1 gram	165	.7902	.0109	.8011	82.97	0.11	14.17	2.75	95	278
½ gram	90	.3958	.0085	.4043	83.51	0.06	14.03	2.40	68	207
½ gram	90	.3670	.0075	.3745	82.78	0.18	14.08	2.96	51	190
¼ gram	50	.2323	.0039	.2362	83.52	0.18	14.00	2.30	15	87
¼ gram	55	.2150	.0032	.2182	83.67	—	14.00	2.33	26	78
¼ gram	50	.2043	.0037	.2080	81.57	0.31	15.49	2.63	43	88
¼ gram	50	.2062	.0033	.2095	82.87	—	14.64	2.49	45	88

seeds removed by the blower were selected by hand and counted. The complete data for samples 4750 and 83419 are given in table 6.

The variances of the data in table 6 by sub-sample weight were calculated and the results are given in table 7. With three exceptions the variance is correlated with sample size; that is, the variance increases as the sub-sample size decreases. The expected variance for ½-gram sub-samples should be twice that of 1-gram sub-samples; for the ¼-gram it should be twice that of ½-gram sub-samples.

The means of percent pure seed, crop seed, inert and weed seed for the three sub-sample weights are so similar that one could easily assume that an equally reliable measure of seed quality could be obtained using any one of the three weights as the quantity for analysis. The variances indicate the same thing. One has to consider, however, that rules of tolerance in the enforcement of seed laws have established certain limits within which seed lots are not considered as mislabeled. The basic sample size is one of about 3,000 particles, and since ¼ gram of redtop seed contains about 2,750 particles, it could be concluded that a ¼-gram fraction, properly obtained and representative, would be adequate. Certainly from the standpoint of time involved it would be of considerable importance if ¼-gram fractions were used instead of 1 gram. Inasmuch as the smaller the sample, the wider the differences to be expected between replicates without showing significant differences, it would seem that ½-gram sub-samples could be used with safety and confidence. *On the other hand, the tolerances for weed seeds, crop seeds and inert as provided in seed testing rules may well be too narrow* (15). This possibility is indicated by the data in table 1.

TABLE 7. VARIANCE AMONG PERCENTAGES OF PURE SEED, CROP SEED, INERT MATERIAL AND WEED SEED OBTAINED FROM REDTOP SUB-SAMPLES SHOWN IN TABLE 6.

Weight sub-sample	No. replicates	Sample 4750				Sample 83419			
		P.S.	C.S.	Inert	W.S.	P.S.	C.S.	Inert	W.S.
1 gram	3	.1263	.0064	.0436	.0124	.1687	.0007	.2534	.0484
½ gram	6	.8388	.0711	.0483	.0072	.2252	.0043	.1967	.0750
¼ gram	12	.4508	.0935	.3010	.0092	1.3300	.0446	.6449	.1056
Means of sub-sample percentages									
1 gram	mean	92.62	.60	6.27	.24	82.62	.12	14.72	2.79
½ gram	mean	92.65	.45	6.76	.14	82.74	.11	14.58	2.58
¼ gram	mean	92.44	.63	6.73	.21	82.36	.20	14.84	2.58

CONCLUSIONS FROM EXPERIMENTS WITH REDTOP SEED

The numerous experiments herein recorded using samples of redtop seed of low, intermediate and high purity show that:

1. The Iowa air blast seed separator when properly operated gives a uniform separation, regardless of sub-sample size, into heavy and light-weight fractions. Repeated blowings of a sub-sample at a given pressure give uniform separation.
2. The standard criterion for measuring air delivery may be valve opening as measured either by millimeters of pressure in the blowing tube or dial reading in degrees. The latter is easier to measure than the former.
3. The dial reading used determines the amount of good seed removed by the air blast, but if it is predetermined by trial it is possible to remove an amount of small consequence in its effect on the percentage of pure seed. It would be possible to standardize the blowing of redtop seed so that the only work required to complete an analysis would be to remove weed seed, crop seed and other foreign material from the heavy fraction.
4. The data confirm previous reports that the use of sub-samples not more than ½ gram in weight will give a reliable measure of the quality of redtop seed lots. The variation among ¼-gram sub-samples is normal, but their use might mean larger differences between replicates than tolerance tables in seed law enforcement provide. The saving in time when even ½-gram portions are used is of considerable importance.

EXPERIMENTS WITH BLUEGRASS

The results of the tests with redtop which showed that constant tube pressure and constant dial opening are about equally dependable criteria when samples of different weight are blown, together with the data published by Porter (6, 7, 8), led to an experiment with bluegrass seed using samples of different bushel weights.

Five samples of seed were selected with bushel weights of 14½, 18, 21, 23 and 30 pounds, respectively. A 1-gram sub-sample was subdivided from each bulk sample and blown in the seed separator according to a graduated scale of tube pressure from low to high. Each sub-sample was blown once and the dial reading recorded for each pressure reading in the tube. The sub-sample from the sample with a bushel weight of 23 pounds was blown once, recombined and blown four times more, making a total of five. The material removed at tube pressures of 6.5, 6.75 and 7.0 mm. was saved and labeled "Inert No. 1," that at tube pressures of 7.25 and 7.75 was labeled "Inert No. 2," and the material removed at the top pressure of 7.75 was labeled "Inert No. 3." All fractions were saved separately, weighed and placed in petri dishes in the germinator to determine the number of sprouts that were produced. The weight of the germinable seeds was then calculated and thus percentage by weight of pure seed in the heavy fraction was determined. All the data which are given in table 8 show that:

1. The dial readings recorded for any given pressure in the blowing tube were highly uniform regardless of bushel weight of sample.
2. The percentages of pure seed obtained by blowing one sub-sample of the 23-pound sample five times were also highly uniform.
3. The total number of germinable seeds removed by blowing sub-samples of different bushel weights by the same schedule was greater for those with a low than with a high bushel weight. The maximum reduction in purity based on removal of germinable seed was 3.17 percent for the 14½-pound seed lot. On the other hand, the removal of such seeds to the inert fraction no doubt removed many more of such similar development that their classification and evaluation by any other known method than uniform air pressure would be extremely difficult and undependable as to uniformity. Furthermore, their retention in the pure seed fraction would have an effect on germination of the pure seed fraction disproportionate to their weight. The high percentage germination of the pure seed fractions from the 14½- and 18-pound seed lots indicates that the blowing schedule was sufficient to provide mature, well-developed seeds. The 21- and 23-pound lots were evidently of low viability. Brown and Porter (1), Leggatt (5) and Porter and Leggatt (9) have shown that up to a certain point which Leggatt has called the "Climax Blowing Point" the removal of light-weight, immature and poorly developed seeds and their retention in the inert fraction give an index value greater than if such particles are retained in the pure seed fraction. This fact is further evident from data in table 9 which show that as the pressure is increased the purity is reduced, but the percentage germination and index value are increased.

To further test the operation of the seed separator three lots of bluegrass seed with bushel weights of 26, 21½ and

TABLE 8. TUBE PRESSURE AND DIAL READING RESULTING FROM BLOWING BLUEGRASS SAMPLES OF DIFFERENT BUSHEL WEIGHTS, TOGETHER WITH PERCENT PURE SEED AND GERMINABLE SEED IN INERT FRACTIONS. IOWA, 1941.

Time min.	Tube pressure mm.	Dial openings in degrees with samples of bu. wts.								
		14½#	18 #	21 #	30 #	1 23 #	2 23 #	3 23 #	4 23 #	5 rep. 23 #
1	6.5	37.2	37.1	37.2	37.2	37.2	37.1	37.1	37.2	37.2
1 Inert 1	6.75	37.3	37.2	37.3	37.3	37.3	37.2.	37.2	37.2	37.4
2	7.0	38.1	37.9	37.9	37.9	37.9	37.8	37.8	37.9	37.9
2 Inert 2	7.25	38.3	38.2	38.1	38.2	38.1	38.0	38.0	38.1	38.1
2	7.50	38.9	38.8	38.8	38.8	38.7	38.5	38.6	38.4	38.4
2 Inert 3	7.75	39.0	39.0	38.9	38.9	38.8	38.8	38.8	38.6	38.9
Percent by weight of inert material blown out and of pure seed										
Inert 1		31.72	16.03	8.72	0.02	6.47	7.26	6.82	6.79	6.73
Inert 2		5.77	2.37	1.96	0.15	1.93	1.06	1.08	1.08	1.57
Inert 3		0.98	1.53	0.68	0.07	0.37	0.45	0.58	0.78	0.60
Pure seed		61.53	80.07	88.64	99.76	91.23	91.23	91.52	91.35	91.10
Number germinable seeds in fractions										
Inert 1		126	19	7	3			13		
Inert 2		58	32	9	5			32		
Inert 3		42	62	6	3			17		
Estimated percent by weight of germinable seeds in terms of total sample weight (Seven germinable seeds per milligram)										
Inert 1		1.77	0.26	0.10	0.04			0.19		
Inert 2		.81	0.44	0.13	0.07			0.46		
Inert 3		.59	0.84	0.09	0.04			0.24		
Total		3.17	1.54	0.32	0.15			0.89		
Percent germination of pure seed		89.5	93.0	75.5	89.0			74.2		

17½ pounds, respectively, were selected. Three sub-samples each of 1 gram and three of ¼ gram were obtained by the Boerner sampler from each lot and blown according to the same schedule as indicated in table 8. Each sub-sample was blown three times at the same schedule and the percentage heavy seed determined each time. The data obtained are given in table 10. The uniformity in percentages of heavy seed from repeated blowings with any given sub-sample was high, likewise the dial readings which measure

TABLE 9. PERCENTAGE PURITY AND GERMINATION AND INDEX VALUES OF BLUEGRASS SEED BLOWN AT DIFFERENT PRESSURES. IOWA, 1940.

Samples	14 mm.			14.5 mm.			15 mm.		
	P. S.	Germ.	I. V.	P. S.	Germ.	I. V.	P. S.	Germ.	I. V.
1	80.3	66	53.0	75.9	76	57.7	71.7	82	58.8
2	—	—	—	77.7	74	57.5	75.5	80	60.4
3	89.9	86	77.3	88.5	88	77.9	87.1	91	79.3
4	83.8	77	64.5	81.8	82	70.8	79.9	84	71.2

P. S. = Pure seed
I. V. = Index value

TABLE 10. PERCENT HEAVY SEED FROM 1-GRAM AND ¼-GRAM SUB-SAMPLES OF BLUEGRASS SEED OF THREE BUSHEL WEIGHTS BLOWN THREE TIMES AT A CONSTANT TUBE PRESSURE. IOWA, 1941.

Sample no.	Bu. wt.		Wt. sub-sample	Highest tube pressure	Dial readings by repetition			Percent heavy by repetition			Mean	Diff. in extremes
					1	2	3	1	2	3		
5973	26#	a	1 gram	7.75	39.2	39.2	39.2	98.85	99.00	99.07	98.97	.22
		b	1 gram	7.75	39.2	39.2	39.2	98.58	98.97	98.63	98.73	.16
		c	1 gram	7.75	39.2	39.1	39.2	99.10	99.17	99.06	99.11	.11
		a	¼ gram	7.75	39.2	39.1	39.2	98.53	98.46	98.72	98.57	.26
		b	¼ gram	7.75	39.2	39.2	39.2	99.38	99.42	99.15	99.32	.27
		c	¼ gram	7.75	39.2	39.2	39.2	99.34	99.42	99.42	99.39	.08
92197	21½#	a	1 gram	7.75	39.2	39.2	39.2	89.01	89.26	89.54	89.27	.53
		b	1 gram	7.75	39.2	39.2	39.2	88.24	88.50	88.28	88.34	.26
		c	1 gram	7.75	39.0	39.0	39.2	88.99	88.99	89.19	89.13	.23
		a	¼ gram	7.75	39.2	39.2	39.2	89.34	89.33	88.91	89.19	.43
		b	¼ gram	7.75	39.1	39.2	39.2	89.09	88.96	88.95	89.00	.14
		c	¼ gram	7.75	39.1	39.0	39.1	89.70	89.70	89.56	89.52	.40
90124	17½#	a	1 gram	7.75	39.1	39.1	39.2	75.93	75.99	76.41	76.11	.48
		b	1 gram	7.75	39.1	39.0	39.1	76.21	76.40	76.44	76.35	.23
		c	1 gram	7.75	39.1	39.2	39.1	76.50	76.70	76.90	76.70	.40
		a	¼ gram	7.75	39.2	39.2	39.2	77.33	77.25	77.27	77.28	.08
		b	¼ gram	7.75	39.1	39.1	39.1	75.35	75.16	75.30	75.27	.19
		c	¼ gram	7.75	39.1	39.0	39.1	79.01	79.26	78.53	78.93	.73

VARIANCES OF DATA IN TABLE 10.

No. and size of sample	No. replications	Mean	Variance	Comparable variances	Range
5973					
1-gram samples	3	98.88	.0817	.3268	.55
¼-gram samples	3	99.09	.2067	.2067	.82
92197					
1-gram samples	3	88.91	.2517	1.0068	.93
¼-gram samples	3	89.24	.0192	.0192	.52
90124					
1-gram samples	3	76.39	.0080	.3520	.59
¼-gram samples	3	77.16	3.3597	3.3597	3.66

the degree of valve opening were as uniform as could be expected with a unit in which the pressure readings could be made accurately to ½ millimeter and the dial readings to ½ degree.

The variances of the data in table 10 show that for samples 5973 and 90124 the variance for ¼-gram sub-samples was greater than for 1-gram samples as would be expected. The reverse occurred for sample 92197. The largest variance was for sample 90124 using ¼-gram portions, yet it is not excessive nor are the differences significant for sub-samples of that size. They are large enough, however, to make their use for practical work open to question.

Following the blowing tests, the inert fraction from the third blowing of each sub-sample was placed in a petri dish and tested for germination. Similarly the heavy fractions were tested by removing 4 × 100 seeds from each one and placing in a petri dish for germination. The data obtained are given in table 11 and they show:

1. Fair uniformity among the number of germinable seeds removed by the seed separator, which when calculated in terms of percent by weight of the total sub-sample indicates a small effect on the percentage of pure seed even for the 17½-pound lot. All sub-samples were not exactly 1 gram or ¼ gram, hence the variation is greater than if a uniform weight had been used.
2. The percentages of germination shown by the pure seed fractions are uniform except for sample 5973, and the germination of the seed from samples 92197 and 90124 is higher than is usually obtained from bluegrass seed lots of that quality. This further emphasizes the importance of removing the poorly developed and immature particles of low vitality. The lack of uniformity in the pure seed fractions from sample 5973 is caused by somewhat unexplainable

TABLE 11. PERCENTAGE GERMINATION OF HEAVY SEED AND NUMBER GERMINABLE SEEDS IN FRACTIONS REMOVED BY BLOWER FROM SAMPLES IN TABLE 10.

Sample no.	Sub-sample wt.	Pct. germ. heavy	Germinable seeds in inert fraction	Pct. by wt. germinable seeds are of total sub-sample *
5973	1 gram a	86.5	8	0.11
26 #	1 gram b	83.5	16	0.23
	1 gram c	82.0	11	0.16
	¼ gram a	86.5	1	0.06
	¼ gram b	91.5	6	0.34
	¼ gram c	85.8	2	0.11
92197	1 gram a	93.5	87	1.24
21½ #	1 gram b	90.3	71	1.01
	1 gram c	93.3	60	.86
	¼ gram a	97.0	11	.63
	¼ gram b	91.8	5	.29
	¼ gram c	95.3	11	.63
90124	1 gram a	90.8	160	2.30
17½ #	1 gram b	93.2	133	1.90
	1 gram c	92.0	143	2.04
	¼ gram a	93.3	31	1.77
	¼ gram b	89.0	24	1.37
	¼ gram c	91.8	24	1.37

* Seven germinable seeds taken as .001 gram or 0.1 percent of 1-gram and 0.4 percent of ¼-gram sub-samples.

yet significant differences noted among the replicates tested from three of the sub-samples. Moisture content of the sand in the dishes may have been too variable, yet not noted at the time of the test. There can be no question about uniform separation by the seed separator in this experiment.

TESTS OF BLUEGRASS SEED WITH A SEED SEPARATOR EQUIPPED WITH VERNIER SCALE ON THE DIAL

Early in the spring of 1942 a new valve with a vernier scale which permitted readings to 1/10 of a degree was obtained and substituted for the old valve. Preliminary trials showed that a satisfactory schedule was as follows:

Dial in degrees	Time in minutes
24.3	4
24.6	2
25.0	2
25.8	1

Two lots of bluegrass seed of 26 and 19 pounds per bushel, respectively, were selected and three 1-gram sub-samples were obtained from each one. Each sub-sample was blown according to the schedule given in the preceding paragraph. The light-weight fraction was examined for firm seeds which were removed, counted and tested for germination. The complete data from these tests are recorded in table 12. They indicate uniformity in percentages of pure seed, inert and weed seed among the sub-samples of each lot as well as small amounts of good seed removed to the inert fraction. The

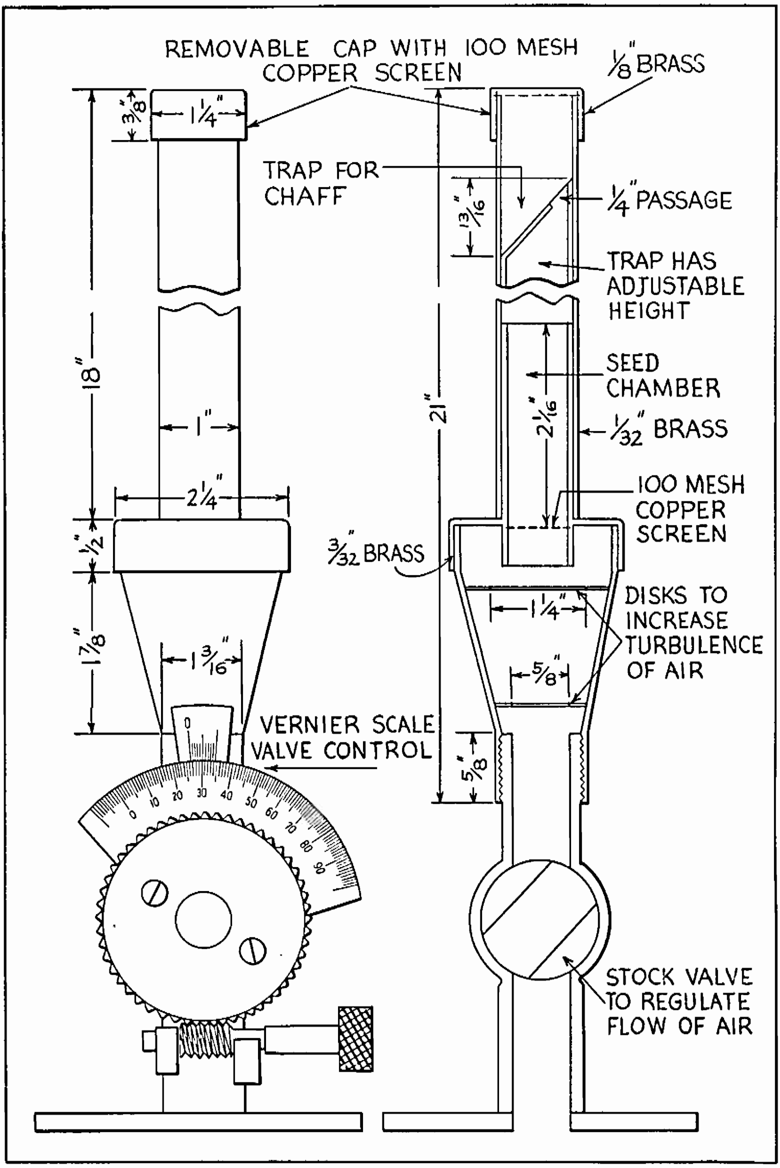

Fig. 1. Iowa air blast seed separator with vernier scale and dial showing exterior view and interior construction. This separator in conjunction with a uniform speed motor and fan constitutes the seed separator unit designed by the Iowa State College Seed Laboratory and constructed by the College Instrument Shop. The vernier scale enables one to open the valve by tenths of a degree. (Reading in figures is 26.5°.)

Detailed procedures for using this seed separator in the analysis of several kinds of grass seed are given in publication No. 9 of the bibliography of this bulletin.

TABLE 12. PERCENTAGES OF PURE SEED BY WEIGHT AND GERMINABLE SEEDS IN HEAVY INERT FRACTIONS OF BLUEGRASS SUB-SAMPLES TESTED IN SEED SEPARATOR WITH VERNIER SCALE ON DIAL. IOWA, 1942.

Sample	Sub-sample	Approx. orig. wt. gm.	Final wt. gm.	Percent			Percent germ.	Seeds in blowings 3, 4		Percent by wt. germinable seeds are of total sub-sample
				P. S.	Inert	W. S.	Pure seed	No.	No. germinable	
19304	1	0.95	0.9131	98.15	1.83	0.02	76	13	2	.03
	2	1.00	0.9999	98 04	1.78	0.18	78	11	2	.03
	3	1.05	1.0686	98.47	1.51	0.02	81	13	2	.03
19306	1	0.95	0.9351	85.20	14.80	—	88	54	17	.23
	2	1.00	1.0140	85.40	14.58	0.02	89	83	25	.36
	3	1.05	1.0972	85.31	14.69	—	89	105	36	.51

Dial and tube readings for above tests

Sub-sample	Dial	Tube pressure	Chamber pressure	Time min.	Bu. wt.	Sub-sample	Dial	Tube pressure	Chamber pressure	Time min.	Bu. Wt.
19304	24.3	6.75	13.5	4	26#	19306	24.3	7.00	13.2	4	19#
	24.6	7.10	14.0	2			24.6	7.20	13.7	2	
	25.0	7.60	14.6	2			25.0	7.60	14.3	2	
	25.8	8.00	15.6	1			25.8	8.10	15.3	1	

pressure readings in the blowing tube and compression chamber are somewhat variable for the two lots of different bushel weight, but these readings cannot be made with the degree of accuracy provided by the vernier scale on the dial which permits reading to 1/10 of a degree. The new dial must be considered more accurate than any previous criteria for measuring air delivery by the seed separator unit.

COMPARISON OF THE IOWA (UNIFORM) AND BINOCULAR METHODS FOR PURITY ANALYSES OF KENTUCKY BLUEGRASS SEED

In 1942 Cullinan (2) proposed what she called the binocular method for determining the purity of bluegrass seed. It consists of thoroughly mixing the bulk sample on a flat surface, then drawing approximately a 1/10-gram fraction from the bulk by means of a spatula. This fraction is then examined under a binocular microscope with strongly reflected light, and separations into pure seed, crop seed, inert and weed seed are made with tweezers.

This method was compared with the official method in 1940 by sending to each of 22 laboratories a 1-gram and a 1/10-gram sub-sample from one lot of seed. Each cooperating laboratory was requested to analyze the sub-samples by the respective methods, and Miss Cullinan tabulated the data in a supplementary report to the participating laboratories. She commented on the results thus, "It was apparent that those who used the newly suggested binocular method and who did so critically and who had adequate equipment to do so, were more uniformly consistent in their results and nearer to the known purity of this stock than were those who made much of the type of blower being used to secure such percentages. All in all there appeared much to commend the binocular method for general use." In connection with the above statement it is important to refer to the data from the referee test and to Cullinan's formal article (2), without which one has only the personal conclusion of the proponent of the method. A summary of the referee test is presented herewith:

No. cooperators 22	Percentages pure seed	
	Official method	Binocular method
Highest	94.5	96.3
Lowest	89.1	87.6
Means	92.0	91.9
Difference in extremes	5.4	8.7
Chi-square	105.5	899.9
Probability	$\angle$ .01	$\angle$ .01

A statistical analysis of the detailed data showed that by either method the probability values were much less than .01, which indicated differences far greater than should be expected from homogeneous samples. For the 1-gram sub-samples it is highly probable that the differences were the result of variable methods of analysis rather than improper sampling because Porter (8) has shown that the Boerner sampler delivers homogeneous sub-samples. The variable results with 1/10-gram sub-samples are probably due both to inexperience with the binocular method and to the greater variance expected among 1/10-gram than among 1-gram portions. Repeated tests by Porter with the uniform method have shown that for lots of bluegrass with a purity percentage of 92 the difference between replicates should not exceed more than 1.5 percent if the sub-samples are uniform. Yet in this referee test the differences are 5.4 and 8.7, respectively, by the official and binocular methods.

The Chi-square value of 899.9 is 10 times more than it actually should be, because only 1/10-gram fractions were used, and the factor in the Chi-square formula (6) is 4.8 rather than 48. The reason for using the larger value is that the binocular method was proposed as a substitute for the official method, and as such the percentages of pure seed should differ at least no more by the proposed than by the official method. A Chi-square value of 89.99 gives a probability much less than .01, which means that the differences are much too large even for such small sub-samples.

An allowance may well be made for inexperience in the use of the binocular method and even in the use of the official method on the part of some of the participants. One might expect, however, that when either method is used with replicate sub-samples by one experienced analyst, uniform results ought to be achieved. The data published by Cullinan (13) are of importance in this connection. She obtained 10 sub-samples each of 1 gram and 1/10 gram from a lot of bluegrass seed. Analyses were made by the official and the binocular methods respectively. In order to properly evaluate the results of the tests and the conclusions drawn, it is necessary to reproduce the purity percentages and to quote from the article in question. The data at the top of page 795 are from the article by Cullinan previously referred to.

Cullinan commented on her results, "No attempt has been made to treat all of this data statistically, especially after it was noted that uniformly the results fell within the latitudes of tolerance allowed. That is to say, the results were just as reliable and consistent as the samples themselves."

It is difficult to interpret the above statement, because in

Sample No.	Pure seed	
	Official method	Binocular method
	%	%
1	90.35	90.71
2	89.70	91.28
3	88.10	91.16
4	88.65	88.92
5	87.90	88.37
6	89.25	89.14
7	89.90	91.33
8	88.00	88.75
9	88.95	90.80
10	89.75	88.92
Maximum T	91.46	92.16
Mean	89.06	89.95
Minimum T	86.64	87.22

the tolerance tables of the Federal Seed Act the maximum allowable difference between two tests of a sample with 89 percent pure seed is 2.83, and for 90 percent pure seed it is 2.64. It should not be assumed that natural variation among replicates should be twice the tolerance or allowable difference between any two replicates. In this connection it is well to emphasize that attempts to measure natural variability by tolerances established for law enforcement are of little value in establishing uniform procedures.

Actually the differences between extremes of the percentages in the data under discussion are 2.45 for the official method and 2.96 for the binocular method. Now a Chi-square test of the data obtained by the official method gives Chi-square equal to 41.52 and a probability of less than 0.01, which means that the differences are greater than should be expected from homogeneous sub-samples if the method of analysis is uniform. If the binocular method is to be used as a substitute for the official method, then a Chi-square analysis of the data from the former method gives Chi-square equal to 71.2 and a probability of much less than 0.01.

Inasmuch as the binocular method employed sub-samples 1/10 the size of those used for the official method, one should expect greater differences by the former than by the latter method. Actually the Chi-square value for the data from the binocular method should be considered 1/10 of 71.2 or 7.12, which gives a probability of .13. This indicates that the percentages of pure seed obtained by the binocular method are therefore more uniform than by the official method in this particular experiment, and the differences among replicates are normal. Yet the difference in extremes is greater than allowed even under the tolerance formula recognized by the Federal Seed Act. The apparent conclusion is that the sub-samples were too small to expect percentage differences of no greater magnitude than when 1-gram sub-samples are used.

TESTS IN THE IOWA LABORATORY

To evaluate the proposed binocular method for analyzing bluegrass seed, samples from nine lots were obtained by the junior authors and analyses were made by the Iowa and binocular methods. The procedure was as follows:

From each lot five sub-samples, each weighing about 1 gram, were obtained by the Boerner sampler. Each sub-sample was analyzed by the Iowa method which employs controlled air pressure as the major basis for separation of pure and impure seed. Following the completion of the analysis the fractions were recombined for each sub-sample, respectively, mixed in the envelope, then poured on a table, remixed with a knife, and a small sample of about 1/10 gram was obtained for the binocular test. In other words, each sub-sample used for the binocular test was drawn from a 1-gram sample on which a test by the Iowa method had been made. The data obtained are given in table 13.

The data in table 13 are worthy of careful study. Analyses by the Iowa method using 1-gram sub-samples of five replicates gave percentages of pure seed reasonably uniform. The Chi-square and probability values indicate that for no lot were the differences significant. It is apparent that the sub-samples were homogeneous. Furthermore, the differences between extremes for each set of replicates were small and indicate that the method of analysis is well standardized.

Analyses by the binocular method using sub-samples from previously determined homogeneous fractions gave larger variations among the respective replicates, as indicated by differences in extremes, than occurred when the Iowa method was used with 1-gram sub-samples. The Chi-square and probability values obtained by analyzing the data from the binocular method indicate a low probability for each lot, which means that the differences are highly significant based on the attempt to substitute the binocular method using 1/10-gram sub-samples for the Iowa method using 1-gram sub-samples. On the other hand, if we wish to measure natural variation among 1/10-gram sub-samples it is necessary to divide each Chi-square value by 10, which gives values of 3.82, 3.96, 1.16, 5.05, 4.19, 3.08, 7.13, 8.68 and 3.13 for lots one to nine, respectively. The corresponding probability values are .43, .42, .88, .28, .39, .52, .12, .07 and .51. We may conclude, therefore, from this experiment that the variations in percentages of pure seed of Kentucky bluegrass when 1/10-gram sub-samples are used are no more

TABLE 13. PERCENTAGES OF PURE SEED OF BLUEGRASS OBTAINED WITH NINE LOTS ANALYZED BY THE BINOCULAR AND THE IOWA METHODS. IOWA, 1941.

Sample no.	Percent pure Binoc.	Percent pure Iowa	Sample no.	Percent pure Binoc.	Percent pure Iowa	Sample no.	Percent pure Binoc.	Percent pure Iowa
1 a	99.1	99.1	4 a	94.2	91.8	7 a	86.9	86.6
b	99.1	99.1	b	93.3	92.4	b	87.5	86.2
c	98.0	99.1	c	91.2	92.9	c	90.9	86.8
d	99.1	99.0	d	94.0	92.3	d	90.7	86.5
e	98.9	99.0	e	94.2	92.4	e	90.2	87.4
Mean	98.84	99.06		93.4	92.36		89.2	86.7
χ^2	38.2	.62		50.5	5.58		71.3	3.3
P	∠.001	.96		∠.001	.22		∠.001	.50
Maximum diff.	1.1	0.1		3.0	0.6		4.0	1.2
2 a	97.8	98.2	5 a	92.2	88.1	8 a	86.2	85.2
b	97.7	98.3	b	89.7	88.4	b	91.9	84.2
c	97.6	98.0	c	92.7	88.2	c	89.2	84.1
d	99.1	98.5	d	90.2	88.9	d	88.4	84.6
e	98.3	98.3	e	90.3	88.4	e	90.0	84.9
Mean	98.1	98.26		91.0	88.4		89.1	84.6
χ^2	39.6	3.7		41.9	1.77		86.8	3.16
P	∠.001	.48		∠.001	.78		∠.001	.52
Maximum diff.	2.5	0.5		3.0	0.8		5.7	1.1
3 a	93.4	91.5	6 a	92.1	89.3	9 a	83.9	77.7
b	93.2	91.1	b	93.1	89.2	b	80.6	79.2
c	92.8	90.8	c	91.3	88.8	c	83.0	79.5
d	93.1	91.3	d	91.2	89.4	d	84.6	78.0
e	94.4	91.2	e	91.2	88.5	e	82.7	78.8
Mean	93.4	91.18		91.8	89.04		83.0	78.64
χ^2	11.6	1.6		30.8	2.8		31.3	6.8
P	∠.02	.80		∠.001	.60		∠.001	.15
Maximum diff.	1.6	0.7		1.9	0.9		4.0	1.8

than may be expected even though they are too great for practical use in seed testing.

The senior author undertook a further evaluation of the binocular method. Two lots of seed, numbers 83064 and 81141 were selected for the study. The plan for the experiment was as follows:

1. Three 1-gram sub-samples were obtained from each lot by the Boerner sampler and analyzed by the Iowa (uniform) method.
2. Six ¼-gram sub-samples were obtained from each lot by the Boerner sampler and analyzed by the Iowa method.
3. Three 1/10-gram sub-samples were obtained by the Cullinan method (using a spatula) and three by the Boerner sampler. All were analyzed by the Iowa method using the air blast seed separator.
4. A total of six 1/10-gram sub-samples were obtained as in 3 above but analyzed by the binocular method using the binocular microscope and tweezers.

The results of the several tests are given in table 14, and the variances of the data are given on page 799. For

TABLE 14. PERCENTAGES OF PURE SEED FROM SUB-SAMPLES OF TWO LOTS OF KENTUCKY BLUEGRASS SEED USING 1-GRAM, ¼-GRAM AND 1/10-GRAM FRACTIONS. IOWA, 1942.*

Group	Sample 83064				Sprouts** 3 and 4	Sample 81141				Sprouts** 3 and 4
	Wt. gram	Description	Percent Pure	Percent Inert		Wt. gram	Description	Percent Pure	Percent Inert	
a	1.0898	Iowa method	92.98	7.02	8	1.0336	Iowa method	83.46	16.30	52
	1.0898	Iowa method	92.95	6.91	10	0.9475	Iowa method	83.85	16.07	39
	1.0898	Iowa method	93.65	6.30	12	1.0061	Iowa method	83.59	16.33	62
b	0.3048	Iowa method	94.49	5.51	3	0.2837	Iowa method	83.58	16.28	8
	0.2450	Iowa method	92.37	7.39	1	0.2783	Iowa method	84.80	15.06	10
	0.2736	Iowa method	94.59	5.41	1	0.2591	Iowa method	85.26	14.74	6
	0.2870	Iowa method	94.04	5.96	1	0.2758	Iowa method	85.06	14.83	12
	0.2926	Iowa method	93.27	6.73	3	0.2561	Iowa method	82.98	17.02	10
	0.2988	Iowa method	92.94	6.99	1	0.2495	Iowa method	85.49	14.11	16
c	0.1163	Iowa method[1]	94.24	5.76	0	0.1296	Iowa method[1]	88.35	11.65	5
	0.1087	Iowa method	91.90	8.10	0	0.0996	Iowa method	86.45	13.55	2
	0.1173	Iowa method	94.72	5.28	0	0.1316	Iowa method	88.91	11.09	3
d	0.0943	Iowa method[2]	92.57	7.22	0	0.1044	Iowa method[2]	85.92	14.08	8
	0.1048	Iowa method	92.56	7.44	1	0.1180	Iowa method	84.41	15.59	4
	0.1010	Iowa method	93.76	6.04	0	0.1379	Iowa method	84.19	15.81	4
e	0.1152	Binoc. method[1]	93.40	6.60	—	0.1406	Binoc method[1]	85.85	13 44	—
	0.1055	Binoc. method	93.56	6.44	—	0.1086	Binoc. method	85.45	14.09	—
	0.1102	Binoc. method	91.65	8.08	—	0.1106	Binoc. method	87.07	12.93	—
f	0.0974	Binoc. method[2]	92.71	7.29	—	0.1012	Binoc. method[2]	83.89	15.91	—
	0.1276	Binoc. method	92.32	7.60	—	0 1373	Binoc. method	81.28	18.72	—
	0.1203	Binoc. method	90.86	9.14	—	0.1256	Binoc. method	82.96	17.04	—

* Iowa method 4, 2, 2 and 1 minutes at increased pressures.
** Inert fractions at third and fourth blowings tested together for germination.
[1] Samples obtained by Cullinan method.
[2] Samples obtained by Boerner sampler.

VARIANCES OF THE DATA IN TABLE 14.

No. samples	No. of replications	Mean	Variance
83064			
a	3	93.1767	.1567
b	6	93.1667	.8043
c	3	93.6200	2.2765
d	3	92.9633	.4760
e	3	92.8700	.5614
f	3	91.9633	.9510
c + d	6	93.2917	1.2303
e + f	6	92.4167	1.0761
c + d + e + f	12	92.8542	1.2576
81141			
a	3	83.63	.4576
b	6	84.53	.8536
c	3	87.90	1.4014
d	3	84.84	.5913
e	3	86.12	.7618
f	3	82.71	1.1666
c + d	6	86.37	4.0077
e + f	6	84.42	4.1424
c + d + e + f	12	85.39	5.2101

sample 83064 the variance for 1-gram sub-samples is less than for any other size, for ¼-gram it is more than four times as great as for 1-gram, but for the four sets of 1/10-gram sub-samples the variance is variable. Taking the 12 ¼-gram sub-samples as a unit, the variance is 1.2576, which is nearly nine times the corresponding value for 1-gram portions, but no greater than to be expected from such fractions. Inasmuch as the variances for groups c + d and e + f are similar we may conclude that it made no difference whether the fractions were analyzed by the Iowa or the binocular methods. On the other hand, if samples c + e are compared with samples d + f it may be noted that the fractions obtained by the Cullinan method are more variable than those obtained by the Boerner sampler. The range in purity for the 12 sub-samples (1/10 gram) is 3.86, and a Chi-square analysis of the data gives a probability much less than .01 if we assume that 1/10-gram sub-samples could be substituted for 1-gram portions. The answer again seems to be in the negative.

Examination of the data for sample 81141 reveals much the same result as for sample 83064. The range in purity for the 1/10-gram portions is 81.28 to 88.91, a difference of 7.63, which is far greater than can be depended on for a uniform standard method. The variance for the 12 sub-samples of approximately 1/10 gram in weight is 5.2101 which is over 10 times that for 1-gram samples. It seems evident, therefore, that the variability among sub-samples of 1/10 gram is no greater than to be expected.

USE OF THE UNIFORM METHOD OF ANALYSIS BY SHIPPERS AND BUYERS OF BLUEGRASS

In the paper by Porter and Leggatt (9) reference was made to comparative tests by different laboratories with several lots of seed. The uniform results obtained were exceptional and far superior to previously conducted tests when the official method is employed.

In 1941 an experiment was undertaken whereby a comparison could be made between samples obtained by the shipper and the buyer of a given lot of seed. Two lots of seed were involved in the experiment. The procedure was as follows: A seed house in Iowa that specializes in bluegrass seed bulked and mixed a quantity amounting to about 300 bags. One of the junior authors visited the seed house and tagged 30 bags from each of which a sample was drawn by a probe. These samples were saved separately and analyzed using a 1-gram sub-sample. In addition the seedsman obtained a large bulk sample (about 10 pounds) from the entire lot by sampling the seed at different places as it was piled in a long rick on the mixing floor. This bulk sample was then passed through a large Boerner sampler, and 12 small samples of about 2 ounces were obtained. Each small sample was retained separately from which a 1-gram sub-sample was obtained and analyzed.

The buyer of this lot was requested to sample each of the 30 bags and to prepare in addition a bulk sample from which 12 smaller portions were obtained. All samples were then submitted to the Iowa laboratory for analysis. The results of tests with the samples from the 30 bags are presented in table 15 and from the 12 small bulk samples in table 16, lot 2.

A second seed house in Missouri was asked to prepare 12 small bulk samples from one lot of bluegrass seed, and the buyer of the seed was requested to do likewise. The samples were then submitted for analysis to the Iowa laboratory, and analyses were made by the uniform method. The results are presented in table 16, lot 1.

A study of the data in table 15 is of interest in that they show, first, how much difference may occur between replicate samples obtained from the same bag and second, how much difference there may be between different bags of seed. It should be emphasized that great care was exercised in the sampling procedures. Each bag was probed at the bottom, center and top and all laboratory analyses were

TABLE 15. PERCENTAGES OF PURE SEED FROM 30 BAGS OF BLUEGRASS SAMPLED BY SHIPPER AND BUYER. IOWA, 1941.

Bag no.	Percentage pure		
	Shipper	Buyer	Diff.
1	87.1	88.8	− 1.7
2	86.6	89.2	− 2.6
3	87.7	87.6	+ 0.1
4	86.3	87.1	− 0.8
5	87.2	85.7	+ 1.5
6	87.6	86.0	+ 1.6
7	86.6	87.8	− 1.2
8	86.5	85.2	+ 1.3
9	85.5	86.3	− 0.8
10	85.4	87.3	− 1.9
11	85.8	86.2	− 0.4
12	89.5	88.2	+ 1.3
13	87.2	86.7	+ 0.5
14	87.1	86.8	+ 0.3
15	83.5	83.5	0
16	86.6	87.4	− 0.8
17	86.6	86.1	+ 0.5
18	87.5	85.9	+ 1.6
19	86.6	87.1	− 0.5
20	87.9	87.2	+ 0.7
21	87.1	87.3	− 0.2
22	86.9	88.8	− 1.9
23	86.4	86.5	− 0.1
24	84.8	86.1	− 1.3
25	87.5	89.4	− 1.9
26	87.4	88.3	− 0.9
27	86.3	87.7	− 1.4
28	86.3	87.1	− 0.8
29	87.7	88.1	− 0.4
30	87.0	88.3	− 1.3
Mean	86.7	87.1	− 0.4
Low	83.5	83.5	0
High	89.5	89.4	+ 0.1

Each percentage is based on 1 gram = 4800 particles of pure seed.

made by a uniform method that has been adequately tested to insure its reliability. The mixing of the bulk lot by the seedsman was also carefully done, and although the differences are greater than should be expected from homogeneous samples, yet the difference between extremes is about 6 percent and possibly may indicate as high a degree of uniformity among bags as can be obtained with chaffy grasses. A study of methods of mixing might well be undertaken to determine if improvement can be made.

The differences between samples submitted by shipper and buyer for any one bag are relatively small, yet for bags 2, 10, 22 and 25 they are slightly more than should be expected from homogeneous lots. The mean percentage pure seed from the 30 bags is 86.7 for the shipper and 87.1 for the buyer, a difference of no statistical significance.

The data in table 16 are largely self-explanatory. They indicate a high degree of uniformity among the 12 samples obtained either by the shipper or the buyer for both lots. The mean percentages of pure seed from the samples sub-

TABLE 16. PURITY PERCENTAGES OF BLUEGRASS SEED SUBMITTED AS 12 REPLICATE SAMPLES BY SHIPPER AND BUYER FROM TWO LOTS. IOWA, 1941.

Lot No. 1				Lot No. 2			
Shipper		Buyer		Shipper		Buyer	
Sample no.	% Pure	Sample no.	% Pure	Sample. no.	% Pure	Sample no.	% Pure
1	76.6	1	74.1	1	86.6	1	87.7
2	75.2	2	75.6	2	87.2	2	88.0
3	76.3	3	74.7	3	86.2	3	87.5
4	76.5	4	76.2	4	87.0	4	87.9
5	75.0	5	75.8	5	87.4	5	88.3
6	74.5	6	75.1	6	86.6	6	87.0
7	76.5	7	74.0	7	87.2	7	88.4
8	76.1	8	74.5	8	87.2	8	87.7
9	76.2	9	74.4	9	87.5	9	87.9
10	75.2	10	76.2	10	86.0	10	87.9
11	76.4	11	74.2	11	86.5	11	86.8
12	76.0	12	74.5	12	86.1	12	88.8
Mean	75.9		74.9		86.8		87.8
Low	74.5		74.0		86.0		86.8
High	76.6		76.2		87.5		88.8
Diff.	2.1		2.2		1.5		2.0
X^2	14.46		14.47		12.69		15.90
P value	.20		.20		.30		.15

mitted by shipper and buyer are significantly different, however, for both lots. For example, the means for lot 1 are 75.9 and 74.9, and the "t" test applied to these percentages gives a value of 3.22 with the least difference for significance at the 1 percent point being 2.8. Similarly the "t" value for a comparison of the means of lot 2 is 4.5, hence the difference is highly significant. It is concluded that for lot 1 the shipper obtained samples with a percentage of pure seed slightly yet significantly higher than obtained by the buyer. The reverse occurred for lot 2. These conclusions are confirmed by a Chi-square test using the 24 samples as a unit for each lot. The Chi-square for lot 1 is 48.8 and for lot 2 it is 66.9. The probability value for each Chi-square is less than .001. On the other hand, if the highest purity value for lot 1 (76.6) were placed on the label and the lowest value (74.0) were obtained from an inspector's sample, the lot would not be considered mislabeled, because the tolerance is 5.15. The same holds true for lot 2, because the tolerance is 2.86 for a high value of 88.8 and a low value of 86.0.

The conclusion that seems justified from this test is that if samples are carefully drawn from a well-mixed lot by both the shipper and the buyer of bluegrass seed the samples may differ significantly, but if the analyses are made by a uniform procedure the differences may be expected to fall within the tolerances allowed for chaffy grasses by the Federal Seed Act.

CONCLUSIONS FROM EXPERIMENTS WITH BLUEGRASS SEED

The numerous tests of bluegrass seed included in this study and representing lots with low, intermediate and high purity have indicated that the Iowa air blast seed separator makes a uniform separation of heavy and light-weight particles for seed of a given weight per bushel and that the preferable basis for regulating the air delivery is dial opening, although pressure in the blowing tube is almost as reliable. A dial with a vernier scale graduated to tenths of a degree is superior to one without the vernier. The schedule to be employed for bluegrass can be easily determined in any laboratory by the use of synthetic samples as proposed by Porter and Leggatt (9). The number of good seeds removed in the light-weight fraction is so small that the effect on the purity is only nominal, yet the removal of such seeds by a uniform method provides a pure seed fraction of such quality that the percentage of germination is raised sufficiently to more than compensate for the reduction in percentage of pure seed. The employment of this particular method based on a new concept of pure seed (9) will give a more correct evaluation of a seed sample than the official method, provide a more standard, uniform procedure and require much less time. It should be borne in mind that the new concept is based not entirely on the botanical definition of a seed (a ripened ovule) but in part on specific gravity of particles which resemble each other so closely that uniform separation by methods other than uniform air pressure is impossible.

The use of a standard method will also aid greatly in the checking of samples submitted from the same lot by shippers and buyers of seed, and thus aid in educating seed merchants to give careful attention to bulking, mixing and sampling methods.

At the present time it would be extremely unwise to depend on 1/10-gram sub-samples for analysis to measure the purity of bluegrass whether analyzed by the binocular or the Iowa method because, although the variation is no greater than to be expected, yet it is too great for use in labeling of seed lots with present tolerance limits. One-fourth-gram sub-samples give smaller differences between sub-samples than do 1/10-gram portions but not as small as with 1-gram sub-samples.

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